Improvements on BBa_K592023 and BBa_K592024 (Blue Fluorescent Protein mTagBFP generators)

Introduction

This characterization on BBa_K592023 and BBa_K592024 (Blue Fluorescent Protein mTagBFP generators) aims to complement the previous characterization submitted by Uppsala Sweden iGEM team in 2011. We used this part as one of the three reporters in our project, Tristable switch, this year. However, when we were finding the characterization data for this reporter, we were surprised that the Uppsala Sweden 2011 did not put a terminator after the mTagBFP CDS. We wondered if the observed fluorescence outputs will be different with versus without a terminator. Therefore, we decided to perform an experiment for comparison. The experimental results showed that the observed fluorescence levels were lowered without a terminator.

Uppsala Sweden 2011 constructed pSB3K3-BBa_K592026, and claimed that it can serve as a reference construct to measure the strengths of other promoters. With BBa_K592026, they measured and reported relative promoter units for BBa_K592003 (P_{cpcG2}), BBa_K592006 (P_{fixK}), BBa_K592008 ($P_{T5\text{-}lac}$). However , given our results, we would like to caution future users in referencing the relative promoter strengths reported by Uppsala Sweden 2011, as their measured expression levels, generated by parts without a terminator, may not fully represent the true strength of the tested promoters.

Results

We hypothesized that the expression level of mTagBFP is affected by the presence or absence of a terminator. To check if that was true, we compared fluorescence outputs from BFP generators with terminator, versus one without a terminator.

An experimental construct was built where the mTagBFP generator (BBa_K592100) was driven by a constitutive promoter (BBa_J23101) and a medium (BBa_B0032). It was compared against its counterpart which harbored a terminator, BBa_B1006 3' to the CDS. Constructs without promoters served as controls for auto-fluorescence.

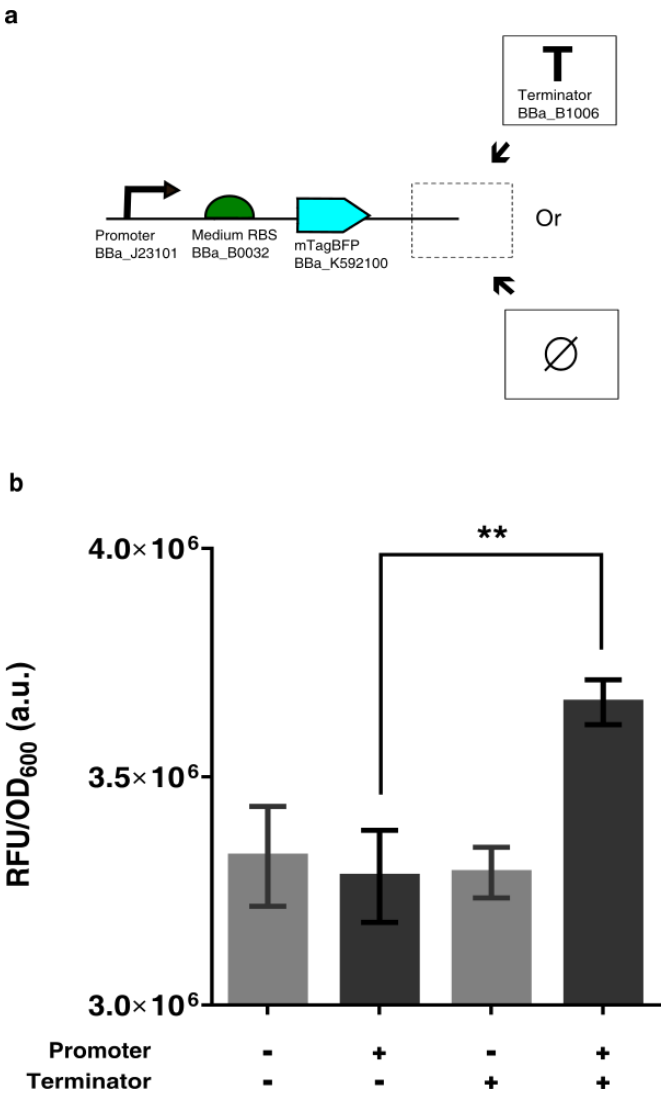

Figure 1. Comparison of the expression levels of a BFP generator with and without a terminator. (a) Circuit diagram illustrates experimental setup of the experiment harboring BBa_B0032. (b) Experimental results. Error bars represent SEM of 3 independent experiments on 3 different days.

Our results showed that the observed blue fluorescence outputs were lowered when the transcription was not properly terminated.

We then performed the same experiment but used BBa_B0034 as the RBS instead, which essentially gave the same result with the difference amplified by the increased translation rate.

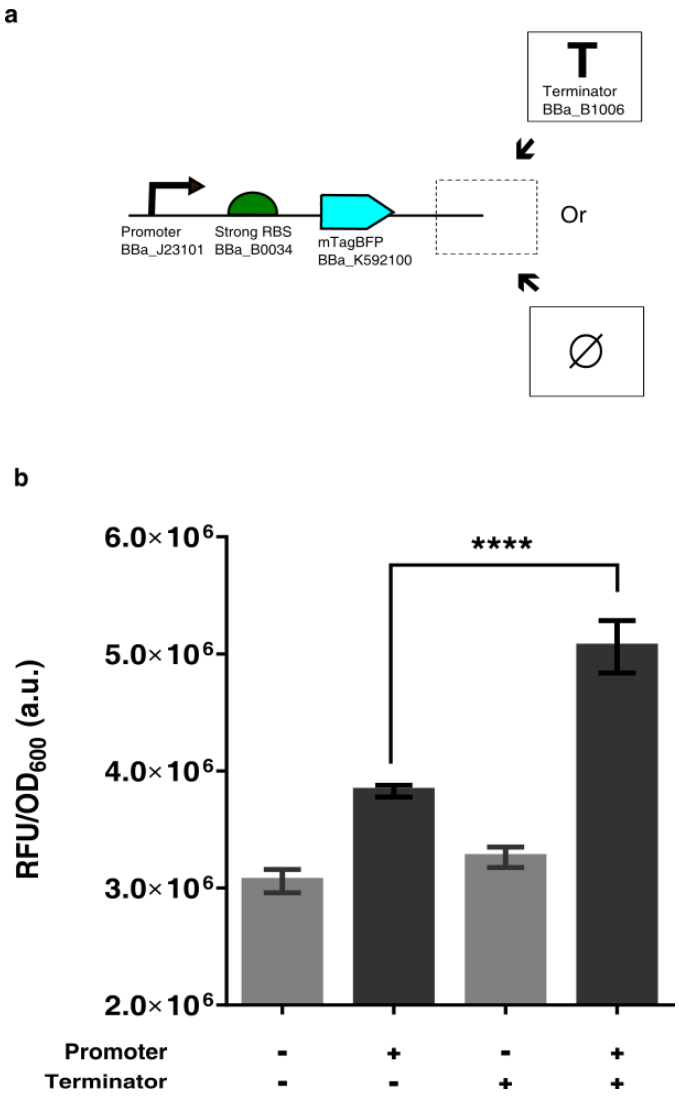

Figure 2. Comparison of the expression levels of a BFP generator with and without a terminator. (a) Circuit diagram illustrates experimental setup of the experiment harboring BBa_B0034. (b) Experimental results. Error bars represent SEM of 3 independent experiments on 3 different days.

Discussion

Our results indicated that the proper expression of mTagBFP requires a terminator, thus the previously submitted parts are translational units only and should not be considered as acceptable alternatives to mTagBFP generators, which have terminators.

We are not sure about the reason behind this phenomenon. It could be an issue related to energy expenditure: transcription run off might have used extra energy resources in the cell, which would normally be available to generate more mTagBFP production. Repeating the above experiment but substituting the strong terminator with terminators of weaker strengths might shed light on our guess.

In this experiment, we have improved BBa_K592023 (BBa_B0032-BBa_K592100) and BBa_K592024 (BBa_B0034-BBa_K592100) by adding a terminator BBa_B1006 3' to these parts. Both of the improved parts, BBa_K1899001 (BBa_K592023-BBa_B1006) and BBa_K1899002 (BBa_K592024-BBa_B1006) were submitted to the Parts Registry this year. With the improved parts, we hope to provide future users with better reporters for their assays. Furthermore, we hope to raise the awareness on the standards of parts measurement through this investigation. We cautioned on the direct use of previous measurement data for promoters generated using BBa_K592023, and we suggest future iGEM teams that undertake measurement experiments of any kind not to omit parts that might appear irrelevant in the measurement cassettes.

Materials and Methods

Strain and Medium

All measurements were done in *E. coli* DH10B (F^- *endA1 deoR*$^+$ *recA1 galE15 galK16 nupG rpsL* Δ*(lac)X74* ϕ80*lacZΔM15 araD139* Δ*(ara,leu)7697 mcrA* Δ*(mrr-hsdRMS-mcrBC)* Str^R λ^-) cells growing in Luria Bertani Broth (1% Tryptone (Sigma #SLBG1640V), 0.5% Yeast Extract (OXOID #LP0021), and 1% NaCl (VWR #14 H 080016) supplemented with 50µg/mL kanamycin (Sigma #SLBH1521V).

Measurement

For each measurement, 3 colonies of each strain were inoculated separately in LB overnight at 37^{o}C with 250rpm shaking. The next day, the culture was diluted 20-fold into a 96 well deep well plate (Sigma#CLS3960) with 475 µl fresh LB medium. The plate was then sealed with paraffin and inoculated at 37^{o}C with 250rpm shaking. OD_{600} was monitored and when most culture in the wells reached OD_{600} from 0.4-0.6, 200 µl of the culture was removed and transferred to a 96 well microtiter plate for measurement. Experiments were repeated for three times on different days as technical triplicates.

Fluorescence measurement was performed using the EnVision Multilabel Reader with the following conditions:

- Absorbance: Photometric 399nm
- Excitation: 399 nm DAPI
- Emission: 456 nm DAPI
- Mirror module: CFP/YFP (428) bottom